ALLOGRAFTING OF CULTURED FIBROBLASTS ON NONHEALING WOUNDS AFTER AUTODERMOPLASTY

D. S. Sarkisov, E. V. Glushchenko, Sh. R. Gurukov, S. S. Morozov, V. P. Tumanov, and N. V. Berezhkov

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Autodermoplasty is one of the most effective methods of treating burns and scar-tissue deformities and contractures arising after burns. Wounds formed after the taking of a split skin graft by means of a dermatome will epithelize in the course of 12-14 days in the absence of complications [2, 3]. However, a serious state of the patients, or infection of the donor areas causes considerable delay of the epithelization of the donor area. This state of affairs prevents the subsequent stages of autodermoplasty from being carried out, it lengthens the patients' stay in hospital, and gives rise to scar changes of the skin. Complications leading to considerable lengthening of the epithelization time are found in 7.8% of patients undergoing autodermoplasty [1]. The use of traditional methods of treatment of nonhealing donor areas by application of oily dressings has proved ineffective, for epithelization is achieved only after the lapse of 1.5-2 months [4].

Connective tissue cells, fibroblasts in particular, are known to influence proliferation of epithelium in vivo [8] and in vitro [9, 10, 11]. A phenomenon of marked stimulation of healing of burn wounds by cultures of fibroblasts has been discovered recently [6]. We have accordingly studied the possibility of using cultured fibroblasts for the treatment of nonhealing donor areas.

EXPERIMENTAL METHOD

A primary culture of fibroblasts was obtained by enzyme treatment of fragments of dermis of patients with burns by means of collagenase ("Flow Laboratories").

To eliminate surface antigens the fibroblasts were subcultured 5-7 times in Eagle's medium (Institute of Poliomyelitis and Virus Encephalitis, USSR), containing 10-15% calf serum, 2% α-glutamine, and antibiotics. The last subculture was carried out in "Heraeus" dishes. At all stages of subculture the fibroblast cultures were examined morphologically, bacterioscopically, and bacteriologically. Surface antigens of the cells at the stages of culture were identified by demonstrating blood group antigens on plasma membranes of human cells in culture [7]. If the bacteriological tests and the tests for blood group antigens were negative, a 3-5-day culture of fibroblasts, having formed a monolayer and possessing high proliferative activity, as confirmed by a high percentage of ^{3}H-thymidine-labeled cells, was transferred to Eagle's medium at 36°C, not containing any additives, and taken to the operating theater (Fig. 1).

Fibroblasts thus obtained were transplanted to nonhealing donor areas of 13 patients, divided into two groups. Group 1 consisted of 7 patients being treated for scar-tissue deformities and contractures, and group 2 of 6 patients being treated in a burns unit for extensive burns of the IIIb-IV degree. After a skin flaphad been taken the wound was immediately covered with a double layer of gauze, impregnated with a solution of the antiseptic chlorhexidine, over which was applied a cotton and gauze dressing with deoxycholate ointment. The cotton and gauze dressing was removed after 24 h and

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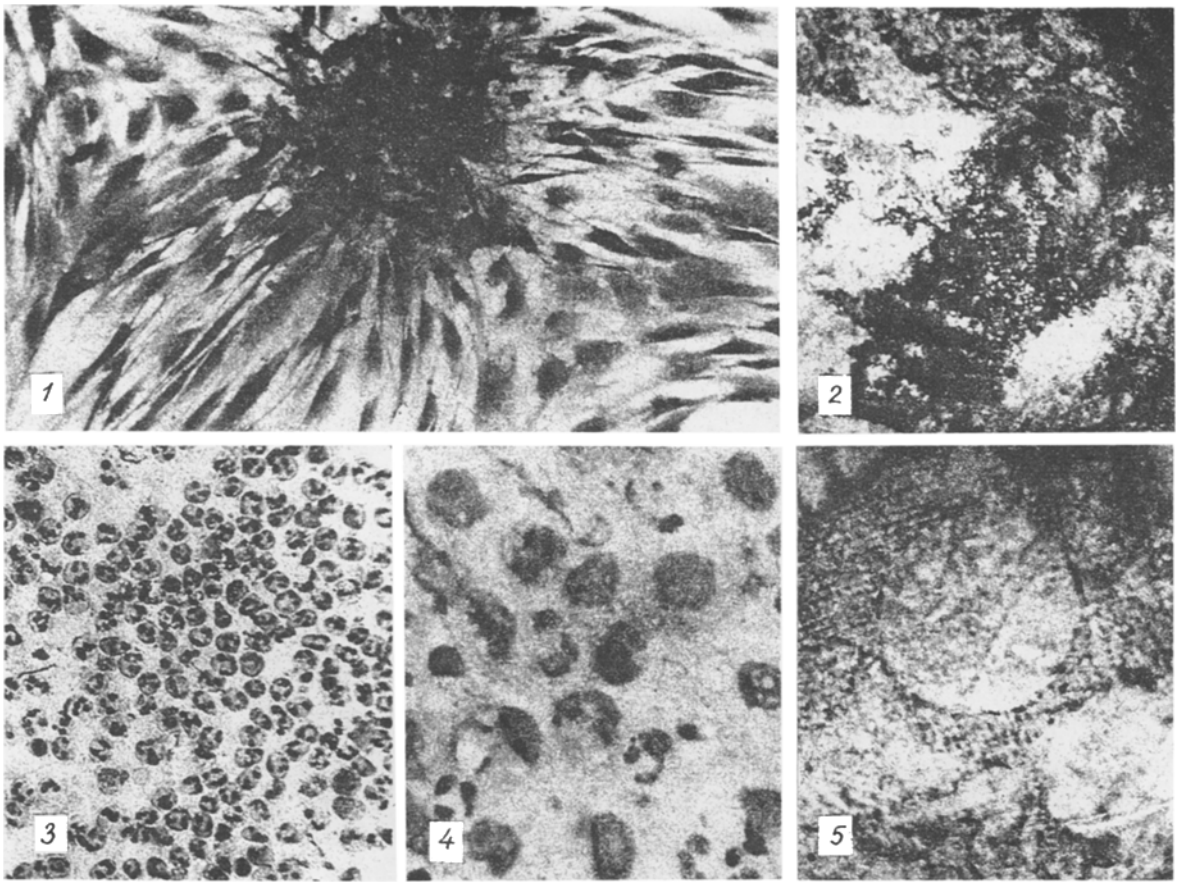

Fig. 1. Monolayer culture of fibroblasts transplanted on wound. Toluidine blue. 200×.

Fig. 2. Indolent wound before transplantation of cultured fibroblasts.

Fig. 3. Predominance of polymorphs in squash preparation from wound before transplantation of cultured fibroblasts. Azure—eosin. 200×.

Fig. 4. Polyblasts, macrophages, and fibroblasts in squash preparation from wound after transplantation. Azure—eosin. 400×.

Fig. 5. Epithelization of wound 7 days after transplantation of cultured fibroblasts.

the gauze remaining on the wound was irrigated with a 5% solution of potassium permanganate. The indication for transplantation of the cultured fibroblasts was absence of epithelization after the specified time. The average time from operation to transplantation in group 1 was 15.2 ± 5.8 days and in group 2 it was 69 ± 33.2 days.

Allografting of the fibroblasts was carried out on the granulating surface of donor areas remaining unhealed after a long time. The graft was covered with paraffin gauze, over which a dry sterile dressing was applied. The average area of the graft was 78.4 cm^2, ranging from 28 to 280 cm^2. The state of the wound in all patients was monitored cytologically and bacteriologically before and after transplantation [5]. Nonhealing donor areas of the same patients, treated with compound ointments, served as the control.

EXPERIMENTAL RESULTS

Nonhealing donor areas of patients of groups 1 and 2, and also wound areas chosen as the control, were characterized before transplantation by the presence of bleeding granulation tissue, covered by fibrin in places. The wound edges had uneven outlines and were hyperemic. In all cases before transplantation no areas of epithelization were observed, or they were single and very small (Fig. 2). Cytologic analysis of squash preparations from the surface of the nonhealing donor areas before transplantation were characterized in all patients by predominance of polymorphonuclear leukocytes. These cells accounted for 79-95% of the total, whereas there were not more than 2% of lymphocytes and very few macrophages. This is the cytologic picture of cellular reactions of inflammatory-regenerative type (Fig. 3). In a squash preparation from a patient of group 1 before transplantation 4% of eosinophils were found, evidence of the presence of an allergic component of the inflammation.

Bacteriologic examination of the wounds of 3 patients of group 1 before transplantation revealed *Staphylococcus epidermidis* with a density of under 100 bacterial cells/cm^2. *Staphylococcus aureus* was found in 3 patients of group 2, but in one of them in a density of over 100 bacterial cells/cm of wound surface. No microorganisms were found in the wound exudate of 4 patients of group 1 and 3 patients of group 2.

The time course of macroscopic and microscopic changes in nonhealing donor areas of patients of groups 1 and 2 after transplantation was similar in character: on the 3rd day after transplantation of fibroblasts a thin transparent film consisting of fibroblasts formed on the wound surface, the granulations decreased in height, bleeding from them ceased, and removal of the dressing was painless. Islands of stratified squamous epithelium appeared on the wound surface. Cytological ezamination of squash preparations showed reduction of the number of neutrophils to 70-57%, polyblasts appeared and numbered 2-7%, and the number of fibroblasts and macrophages increased to 10% or more. In the patient with an allergic component of inflammation the number of eosinophils increased to 7% by the 3rd day, but the islets of epithelization were smaller. The cell composition of the squash preparations by the 3rd day was mixed regenerative-inflammatory or purely regenerative in character (Fig. 4). Bacteriologic tests were negative in 12 patients. Only in 1 patient, with the highest positive seeding rate before transplantation, suppuration of the graft was observed on the 3rd day. *Staph. aureus* was isolated from the wound exudate of this patient in a density of 10 bacterial cells/cm^2 of wound surface. This patient's donor area was subsequently treated with oily dressings.

The wounds of 5 patients of group 1 were completely epithelized 6-8 days after transplantation (Fig. 5), with the patient with an allergic component of inflammation as the exception: epithelization of the wound took place slowly and in the form of discrete islands. The average duration of the process of epithelization in patients of group 1 was 6.8 $\pm$ 1.2 days, and the longest duration was 10 days. The times of epithelization in patients with burns were 7.6 $\pm$ 1.2 days. Single fibroblasts and stratified squamous epithelial cells were identified in squash preparations at these times and no microflora could be found in the wound exudate.

The cytology of the wound in the control group was characteristically inflammatory or mixed inflammatory-regenerative in type; even after 3 weeks adequate epithelization could not be observed.

The investigation thus showed that the use of cultured fibroblasts to treat donor areas remaining unhealed for a long time is highly effective. Transplantation of the cells led to rapid epithelization of the wounds even in badly burned patients. Microbial contamination of the wounds before transplantation by *Staph. epidermidis* and *Staph. aureus* in a density of under 100 microbial cells/cm^2 of wound surface did not impair the results obtained by the use of cultured fibroblasts. However, a density of microbial cells of over 100/cm^2 of wound, as practical experience has shown, is a contraindication to the use of grafts, for they can cause suppuration. It must be emphasized that, even though allografts were used, we did not observe a single case of graft rejection. Inspection of the state of the donor areas in the late stages (after 2-5 months) showed that the state of the epithelized wounds was good. The absence of a rejection reaction can be attributed, in our view, to elimination of the surface antigens of the cells during subculture.

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CORRELATION BETWEEN MAST-CELL AND LEUKOCYTIC ACTIVITY AND PERMEABILITY OF MESENTERIC VENULES IN RATS WITH EXPERIMENTAL PERITONITIS

K. T. Zaitsev

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Disturbance of permeability of the walls of microvessels, manifested as increased outflow of protein into the tissues through the walls of venules, is one of the most obvious signs of inflammation in the early stages of its development [2]. An important role in the increase of permeability is played by mast cells and also, perhaps, by leukocytes, a change in the functional state of which in the early phase of inflammation is a well known phenomenon [13]. These cell groups produce factors which are interpreted as the most active "permeability mediators." They include histamine and active forms of oxygen [12, 14].

The aim of this investigation was to assess dependence of increased protein transport from blood into tissue in the region of inflammation on the level of functional activity of mesenteric mast cells and on adhesion of leukocytes circulating in the mesentery.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing about 300 g. Experimental peritonitis was induced in the animals under ether anesthesia, by irrigating the surface of the mesentery with a filtrate of contents of the large intestine [3]. The peritoneal cavity was closed without drainage. Observations began 1, 2, 4, and 8 h after induction of peritonitis. The state of the mast cells and leukocytes was analyzed by intravital microscopy: under pentobarbital (50 mg/kg) anesthesia laparotomy was performed, and a loop of small intestine with segments of the mesentery were laid on a special stage, irrigated continuously with warm (37°C) Hanks' solution (pH 7.4), and examined in a "Leitz" intravital microscope under ordinary and luminescent illumination. Adhesion of leukocytes to the luminal surface of the venular endothelium was evaluated by counting the number of cells in a 100-μ length of a vessel about 50 μ in diameter [4]. Next, the mast cells

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